IRB EFFICACY REVIEW

PRODUCT NAME:	ALPHACHLORALOSE TECHNICAL
PRODUCT FILE SYMBOL:	89670-E
REGISTRANT:	Lodi Group. Grand Fougeray, France
DATE COMPLETED:	9/4/2014
DP NUMBER:	421801
DECISION NUMBER:	474730
DATE OF SUBMISSION:	7/2/201 (received 7/2/2014, sent for review 7/29/14)
ACTIVE INGREDIENTS:	Alpha-chloralose
FORMULATION:	90.27% Alpha-chloralose
TYPE OF PRODUCT:	Rodenticide
PURPOSE:	New product and new active ingredient reregistration
DATA MRID NUMBER:	494231-01
GLP CLAIMED:	No
TEAM REVIEWER:	Gene Benbow
EFFICACY REVIEWER:	William W. Jacobs, Ph. D. 9/4/2014
SECONDARY REVIEWER:	Jennifer Gaines, M.S. 9/4/14

BACKGROUND

This product is a 90.27% Alpha-chloralose technical formulation intended to be registered

> FOR FORMULATION ONLY INTO END-USE RODENTICIDE BAITS AS DESCRIBED IN THE DIRECTIONS FOR USE.

There currently are no Alpha-chloralose formulations registered as pesticides in the U.S. Consequently, this compound is considered to be a new active ingredient.

On 2/1/13 and on behalf of the Lodi Group, ToXcel Toxicology & Regulatory Affairs submitted an application to register this manufacturing-use product Alpha-Chloralose Technical (89670-E). On 5/15/13 and subsequently, EPA informed ToXcel of various deficiencies in that application and in the application to register a "4.45%" Alpha-chloralose paste bait formulation as an end-use product (89670-R). On 8/7/2013, I completed a review of efficacy-related materials that had been submitted for 89670-R. I concluded that the efficacy data package remained deficient.

One of the efficacy data deficiencies noted in that review was the lack of acute toxicity data on the house mouse, the sole target species proposed to be claimed for 89670-R. On 7/2/14, ToXcel submitted a report of an acute toxicity study and linked it to the technical product, 89670-E. This review assesses that study.

In addition to the study report itself, the review package also included the items identified below.

1. ToXcel's letter of submission, dated "July 2, 2014"
2. a completed application form (8570-1), also dated "July 2, 2014"
3. a TRANSMITTAL DOCUMENT for the submission of 7/2/14

To support registration of a new active ingredient for controlling house mice in the U.S., efficacy of the active ingredient and end-use products containing it must be established on wild-type house mice (*Mus musculus*) from U.S. populations.[1] Claims for controlling house mice with new active ingredients are to be substantiated through submission of laboratory and field efficacy data on wild-type house mice. For a rodenticide claimed to be effective when used in bait form, it may become possible, eventually, to substitute laboratory-strain (preferably Swiss-Webster) house mice for wild-types as subjects in laboratory efficacy trials. However, such substitution should be permitted only after similar results in laboratory efficacy trials on the same bait formulation, using the same acceptable methodology, have been obtained with wild-type and laboratory-strain house mice.[2]

The suitability of an active ingredient source product as a rodent control agent, which is what is being proposed for 89670-E, typically is established once at least one end-use product (perhaps 89670-R) or formulation made from it has been shown to meet the applicable efficacy criteria for U.S. registration. For reasons discussed in the efficacy review of 8/7/13 for 89670-R, none of the efficacy-related reports previously submitted for that product of 89670-E was accepted.

The European Commission (EC, 2008) assessed possible rodenticidal uses of Alpha-chloralose in European Union member states and reached the conclusions quoted below (from page 17).

> Alphachloralose shall be included in Annex I to Directive 98/8/EC as an active substance for use in product-type 14 (Rodenticide) subject to the following condition:
>
> Minimum purity of 825 g/kg in the biocidal product as placed on the market;

Alpha-chloralose has a long history of use as an anesthetic and, in countries other than the U.S., as a rodenticide (e.g., Gerace, *et al*, 2012; Brooks, 1973).[3] In the U.S., there is some use of Alpha-chloralose as an anesthetic to facilitate capturing of birds (e.g., Smith, 2004 a, b). Material Safety Data Sheets (MSDSs) for Alpha-chloralose list acute oral LD_{50} values of 200 mg/kg of body weight for "mouse" and 400 mg/kg bwt for "rat" (e.g., Fisher Scientific, 2009; MP Bio, 2005). LODI's MSDS (LODI, 2011) for "**BLACK PEARL PASTE**" mentions a "**Rat oral LD50**" of 341 mg/kg but does not include a figure for house mice.

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[1] Alpha-chloralose has been used as a rodent-control agent at various times and in various places around the world over the past 4-5 decades. Brooks (1973) mentioned it in his review of commensal rodents and their control. As it is not registered for that purpose in the U.S., however, Alpha-chloralose it is treated as a new active ingredient under FIFRA.

[2] This approach has been followed by EPA since at least 1974 and has been summarized in various documents, including recently by Jacobs (2011).

[3] Citing a general reference document, Gerace, *et al* (2012), state that, for humans "The oral toxic dose of chloralose is approximately 1 g in adults and 20 mg/kg in infants." To ingest 1 g of Alpha-chloralose, a human adult would have to consume 25 g (0.88 oz.) of a 4% Alpha-chloralose bait. There have been "successful" adult human suicides involving Alpha-chloralose (e.g., Gerace, *et al*, 2012). A 10 kg (22-lb) infant would receive a 20-mg/kg dosage by ingesting 5 g of a 4% Alpha-chloralose bait.

DATA SUMMARY

Formulation

See "CONFIDENTIAL ATTACHMENT" to this review.

Efficacy Data

The report of acute toxicity trials submitted on July 2, 2014, is cited and discussed below.

Bureau, M. (2014) Chloralose: evaluation of the effects after acute oral administration and at 2 different housing temperatures (22° C and 30° C) in albino house mouse. Unpublished Study Report, Final Version, Biotrial Pharmacology, Non Clinical Pharmacology Department, Rennes Cedex, France, 26 pp.

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Bureau (2014) reports that this trial "is a pharmacological study and thus does not require GLP status." Bureau adds that

> analysis and reporting were carried out according to Biotrial Standard Operating Procedures (SOPs), in line with GLP principles.

Efficacy studies and toxicology studies used to support applications for pesticide registrations in the U.S. should be conducted according to EPA's requirements for Good Laboratory Practices. That having been noted, the conduct and results of this trial are reviewed.

Bureau identifies the test substance used in this study as powdered material from "Chloralose (batch PP/ALPHA (B)/11/08". This material was "freshly suspended in 1% Methocel"[4] and administered by oral gavage in a volume of "10 mL/kg body weight", equivalent to 0.25 ml for a 25-g mouse.

"Chloralose" at times is used synonymously with "Alpha-chloralose", although that material typically co-occurs with much lower amounts of Beta-chloralose, which reportedly is inert as a rodenticide. It is not clear from Bureau's report whether the "Chloralose" dosages indicated represent Alpha-chloralose alone or the mixture of Alpha-chloralose and Beta chloralose.

Test subjects were not wild-type house mice but rather were of the "RjOrl:Swiss (CD-1)" laboratory strain. Thirty males and 30 females were used. Starting body weights for males reportedly ranged from 26 to 33 g, whereas females weighed 21 to 25 g.[5] Mice were housed in single-sex subgroups of 5 animals each throughout the study. Cages had polypropylene bottoms that were 1032 cm^2 in area.[6] Study personnel used "indelible markers" to mark the tails of mice so as to be able to identify and distinguish individuals.

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[4] This material is identified further in the Bureau (2014) report as "Methylcellulose (batch No. SLBB2809V ref. M0262)" obtained from "Sigma Chemical Co. (Saint Quentin Fallavier, France)".

[5] Bureau (2014) notes that, as one of two "**Deviations to Study Plan**", "Weight ranges for male and female mice were changed: 21-35 g and 17-25 g, instead of 21-24 g and 17-20 g, respectively." According to the author, that change "did not affect the integrity or validity of the results of the study." All of the weight ranges indicated here and the initial weights of test subjects were within the 15- to 35-g range permitted for laboratory efficacy trials in OPP's Protocol 1.210, which describes a choice-feeding method for assessing acute rodenticide baits for effectiveness against house mice.

[6] This cage-bottom area is below ≥2000-cm^2 range indicated in OPP's protocols (e.g., Protocol 1.210) for screening rodenticide baits for efficacy against house mice if the animals are group-caged. Having single-sex subgroups of 5 mice each also is permitted by those protocols. Group-caging of subjects used in acute oral LD_{50} trials is unusual, however, and might have been employed in this case due to a misinterpretation of what was needed for this type of

Laboratory environmental conditions were set to a 12-hr/12-hr light/dark cycle, and to provide 15-20 air replacements per hour and a relative humidity of "55±10%". Room temperature was controlled as an independent variable in this study, with one half of the mice (3 groups of 5 females and 3 groups of 5 males) being kept at "22±2°C" (70.6±3.6°F) and the other half at "30±2°C" (86.0± 3.6°F). Mice were housed under these "environmental conditions for at least 5 days prior to experimentation."

For each of the temperature conditions (22±2°C and 30±2°C) one group (comprised of a 5-male subgroup and a 5-female subgroup) was gavaged at a dosage of "Chloralose (400 mg/kg, po)". That dosage and subsequent dosages "were chosen by the sponsor according to the known pharmacological profile of this compound" (see BACKGROUND above). The dosages selected subsequently were "Chloralose (200 mg/kg, po)" and "Chloralose (300 mg/kg, po)". Bureau does not mention inclusion of a vehicle-only group or any other sort of control group.

Dosage	Sex	Time to 100% of mice "sleeping"	No. Killed	No. Surviving	Percent Killed
		Mice Kept at 22+/-2°			
200 mg/kg bwt	Females	60 minutes	1	4	20%
"	Males	"	0	5	0%
"	Both	"	1	9	10%
300 mg/kg bwt	Females	30 minutes	4	1	80%
	Males	"	1	4	20%
	Both	"	5	5	50%
400 mg/kg/bwt	Females	15 minutes	5	0	100%
	Males	^'	1	4	20%
	Both	"	6	4	60%
		Mice Kept at 30+/-2°			
200 mg/kg bwt	Females	60 minutes	0	5	0%
"	Males	"	0	5	0%
"	Both	"	0	10	0%
300 mg/kg bwt	Females	30 minutes	1	4	20%
	Males	"	0	5	0%
	Both	"	1	9	10%
400 mg/kg/bwt	Females	15 minutes	5	0	100%
	Males	^'	2	3	40%
	Both	"	7	3	70%

study. Group-caging would have afforded the mice opportunities to huddle together, which might have been helpful to them following dosage with Alpha-chloralose. However, the first mice that "came to" would have been afforded and opportunity to cannibalize those that had not. Cages used in trials conducted according to OPP's laboratory efficacy protocols for trials involving house mice ares supposed to be "solid-bottom all metal cages" (Protocol 1.210, paragraph. 3.1).

Primary study results are summarized in the table above. All mice reportedly were "sleeping" (presumably meaning anesthetized) following administration of the test material, with the length of the latent period before that state was achieved being related to strength of dosage. Dosage, room temperature, and gender all seemed to affect whether animals died or revived. For all temperature and dosage combinations for which there were any mortalities at all, more females than males died. Among groups housed at the same temperature, the 400-mg/kg dosage killed more mice than did the 300-mg/kg dosage which, in turn, killed more mice than did the 200 mg/kg dosage. Lumped across dosages and genders, 12 of 30 (40%) kept at 22±2°C died whereas 8 of 30 mice (26.7%) kept at 30±2°C died. The room-temperature effect occurred at the intermediate dosage (300 mg/kg of body weight) where 5 of the 10 mice kept at 22±2°C died and 1 of the 10 mice (10%) kept at 30±2°C died.

Bureau reports LD_{50} figures of 300 and 363 mg/kg of body weight, for house mice of the tested strain when housed at 22±2°C and 30±2°C, respectively. These figures pertain to the data obtained with both sexes combined. Bureau does not report separate figures for females and males, despite the apparent difference in sensitivity between genders. None of the dosages administered killed half of the males kept at 22±2°C or 30±2°C. If a rodenticide is to be more toxic to one sex than to the other, it is better for control programs if females are more sensitive than males. That is because the intrinsic rate of population increase for polygynous species is directly related to the number of reproductively capable females.

Bureau reports initial body weights for all subjects and body weights 24 hours following dosage for the mice that did not die as an apparent result of gavage with the test material. All 7 mice that survived administration of the 400-mg/kg dosage lost weight (1-5 g), regardless of whether they were kept at 22±2°C or 30±2°C. The 14 survivors of the 300-mg/kg dosage included 11 that lost weight (1-3 g) and 3 that maintained their initial weight. The 19 survivors of the 200-mg/kg dosage included 6 that lost weight (1-2 g), 9 that maintained their initial weight, and 4 that gained (1 g each). These apparently dose-related results probably were related to the period of time over which survivors were anesthetized. Latency to group anesthetization reportedly was dose related, and such also might have been the case for time to full behavioral recovery.

Based upon the results reported by Bureau, the LD_{50}s for females alone would be below 300 mg/kg of body weight at ambient temperature of 22±2°C and somewhere between 300 and 400 mg/kg at an ambient temperature of 30±2°C. Bureau's data do not permit estimations of LD_{50} figures for male house mice, but it seems clear enough that the values that might be obtained with additional experimentation would be in excess, perhaps well in excess, of 400 mg/kg of body weight for ambient temperatures of 22±2°C and 30±2°C. To self-administer a dosage of 400 mg/kg, a 25-g mouse would have to ingest 10 mg of Alpha-chloralose. To do that, the mouse would have to consume 0.25 g of a 4% Alpha-chloralose bait. If the 25-g mouse were male, more bait than that likely would be needed to ingest an LD_{50} dosage.

The results obtained by Bureau (2014) were not consistent with the LD_{50} figure of 200 mg/kg bwt for "mouse" that appears on the MSDS documents cited in this review that provide a "mouse" figure.

In this trial, Bureau killed some subjects that received dosages of test material by oral gavage. The test organisms used were from a laboratory strain of the house mouse rather than the wild-type house mouse, which is the pest for which control is proposed for the pending end-use Alpha-chloralose product 89670-R. Wild-type house mice tend to be smaller than laboratory strains. Thus, wild-type house mice would be expected to have higher surface-to-volume ratios than would laboratory strains of the same species. As Alpha-chloralose kills via induced hypothermia, wild-type house mice might be more sensitive to it than laboratory strains would be because of differences in surface-to-volume ratio, although other factors also could be involved. Within strains, smaller and younger animals might be more sensitive, although neonatal mice might have adaptive mechanisms to resist hypothermia. As there was no overlap in initial body weights of male and female subjects in Bureau's study, it is possible that the apparent gender difference in sensitivity to Alpha-chloralose was more related to body weight than to sex. Within subgroups (eliminating dosage, sex, and room temperature as factors), however, there was no obvious relationship between initial body weight and survival in Bureau's study.

To the extent that the results reported by Bureau are relevant to use of Alpha-chloralose to control house mice, it appears that free-living house mice would have to reliably self-administer dosages of Alpha-chloralose via bait consumption well over 400 mg/kg bwt in order for this compound to function effectively as a rodenticide at almost any environmental temperature.[7]

CONCLUSIONS

1. The report by Bureau (2014, MRID No. 494231-01) describes trials intended to demonstrate the acute oral toxicity of Alpha-chloralose to the house mouse and, thereby, to fulfill one of the efficacy data requirements for registering this compound in the U.S. as a new active ingredient for controlling house mice. Bureau (2014) also used environmental temperature as in independent variable, running similar testing with animals kept at room temperatures of 22±2°C and 30±2°C. There were several deficiencies in the conduct of the study. These deficiencies are listed below.

    a. Test subjects were from a laboratory strain of house mice rather than the actual pest organism – wild-type *Mus musculus* from U.S. populations.

    b. There were no control groups (vehicle-only or even housing-conditions-only).

    c. Mice were group-cage rather than caged individually.

    d. The sequential testing procedures employed were not continued to permit determination of within-gender LD values despite the sex difference in response that was obvious from the first dosage administered.

2. Taken at face value, the results reported by Bureau (2014) suggest that dosage, gender, and prevailing temperature affect the sensitivity of house mice to Alpha-chloralose. Under the conditions (22±2°C and 30±2°C) tested, the effect of temperature appeared to be important only at the intermediate dosage used (300 mg/kg of body weight). The acute oral LD_{50} values determined for those temperatures (300 mg/kg at 22±2°C and 363 mg/kg at 30±2°C) reflect the pooled results for both sexes. Based upon the results reported by Bureau, the LD_{50}s for females alone would be below 300 mg/kg of body weight at ambient temperature of 22±2°C and somewhere between 300 and 400 mg/kg at and ambient temperature of 30±2°C, respectively. Although Bureau's data do not permit estimations of LD_{50} figures for male house mice, the values that might be obtained with additional experimentation likely would be above to above 400 mg/kg of body weight for ambient temperatures of 22±2°C and 30±2°C. To self-administer a dosage of 400 mg/kg, a 25-g mouse would have to ingest 10 mg of Alpha-chloralose. That would require the mouse to consume 0.25 g of a 4% Alpha-chloralose bait. If the 25-g mouse were male, consuming more bait than that likely would be needed to ingest an LD_{50} dosage (which would not kill all male mice that ingested it).

3. Although a more appropriate and more thorough study should have been commissioned, additional acute oral toxicity data for the house mouse will not be required at this time.

REFERENCES

Brooks, J.E. 1973. A review of commensal rodents and their control. Critical Reviews in Environmental Control, 3:4, 405-453.

EC. 2008. Alphachloralose Product-type 14 (rodenticide). Assessment Report, Annex 1 PT, Directive 98/8/EC concerning placing biocidal products on the market, May 30, 2008, European Commission, 61 pp.

[7] The "LD_{99}" would be the minimum dosage needed to reliably kill mice in a control program, and that figure would be well above Bureau's LD_{50} figures at the tested room temperatures and perhaps under cooler conditions as well.

Fisher Scientific. 2009. Alpha-Chloralose. Material Safety Data Sheet, Created September 2, 1997; Revised, Revision #10 dated July 20, 2009, 4 pp.

Gerace, E., Ciccotelli, V., Rapetti, P., Salomone, A., and Vincenti, M. 2012, Distribution of chloralose in a fatal intoxication. Journal of Analytical Toxicology, 00; 1-5 (doi:1093/jat/bks040)

Jacobs, W.W. 2011. Efficacy issues regarding control of commensal rodents in the U.S. using registered rodenticides. Registration Division, Office of Pesticide Programs, Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C., 62 pp.

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MP Bio. 2005. Alpha-Chloralose reagent grade. Material Safety Data Sheet, MP Biomedicals Australasia Pty Limited, Seven Hills, NSW, Australia, 6 pp.

Smith, M.A. 2004a. Capturing problematic urban Canada geese in Reno, Nevada: goose roundups vs, use of Alpha-chloralose. Proceedings: 21st Vertebrate Pest Conference (Timm, R.M. and Gorenzel, W.P., eds.), University of California at Davis, CA, 97-100.

Smith, M.A. 2004b. Capturing nuisance urban Canada geese using the bird immobilizing agent Alpha-chloralose in Reno, Nevada: what we have learned. Proceedings: 21st Vertebrate Pest Conference (Timm, R.M. and Gorenzel, W.P., eds.), University of California at Davis, CA, 101-103.